Breeding and Selection of *Prunus* Rootstocks at the Aula Dei Experimental Station, Zaragoza, Spain

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Abstract

The selection and study of the performance of rootstocks for stone fruit species has been and remains one of the main tasks of the Department of Pomology of the Aula Dei Experimental Station. Initial selection was made from spontaneous *Prunus* germplasm, mainly open-pollinated, growing in Spain. In the first years, this selection programme included rooting ability as an important trait. Other main objectives were tolerance to calcareous soils and graft compatibility with a wide range of cultivars in the nursery. Elite candidates during these initial screens were virus tested, propagated and tested with a limited number of scion cultivars. Trees were planted in the field and grown under normal management techniques for the area, with records kept for several years, in which growth and fruiting were evaluated. Following this long process, several clonal rootstocks have been released and some of them are already under extensive commercial exploitation: the peach × almond hybrids 'Adafuel' and 'Adarcias', the pollizo plum 'Adesoto', the plum rootstock 'Adara' and the myrobalan 'Ademir'. Currently, the *Prunus* breeding programme is mainly directed to obtaining new stone fruit rootstocks from hybridisation within *Prunus* related species and selecting for specific adaptation to unfavourable Mediterranean environments. Good adaptation to replant sites, tolerance to chlorosis and waterlogging, resistance to nematodes and other soil borne pathogens, and graft compatibility are considered priority traits.

INTRODUCTION

The selection and study of the performance of rootstocks and varieties of stone fruit species has been, and continues to be one of the priority objectives of the Department of Pomology of the Aula Dei Experimental Station (EEAD-CSIC), since it was founded in 1950. These studies initiated with surveys and establishing *Prunus* germplasm collections to determine the variability in the most cultivated stone fruit species that existed in Spain (Herrero et al., 1964).

In general, rootstocks belonging to the *Prunus* genus are graft compatible with other stone fruit species within *Prunus* L. This polyvalent feature increases the utility of the rootstocks selected, and allows species with low intra-specific variability to be grown under limiting pathological and soil conditions.

Regarding rootstock selection, research has been carried out according to the different cultivar and/or rootstock species:

1) Plum rootstocks (mainly *Prunus domestica* L., *P. insititia* L. and *P. cerasifera* Ehrh.) for peaches, plums and prunes, apricots and almonds.
2) Inter-specific hybrids of peach × almond (*P. amygdalo-persica*) for peaches and almonds.
3) Cherry rootstocks (*P. cerasus* L. and *P. cerasifera* Ehrh.) for sweet and sour cherries.

Selection of Rootstocks from Different Plums

Plum rootstocks are more tolerant to heavy soils and waterlogging problems than other species of *Prunus* L., a fundamental reason for their use (Bernhard and Grasselly,

1959; Rowe and Catlin, 1971; Salesses and Juste, 1970; Bernhard et al., 1979). Tolerance to iron-induced chlorosis in calcareous soils and graft compatibility with different species have also promoted the use of these rootstocks (Moreno et al., 1995a; 1995b; 1995c). Furthermore, some are claimed to have earlier onset of production and better fruit quality (Bernhard and Grasselly, 1959; Loreti and Massai, 1990; Moreno et al., 1990).

This group of rootstocks includes the Myrobalans (*P. cerasifera*), 'Pollizos de Murcia' (*P. insititia*) and other slow-growing plums (*P. domestica*). In addition, there are numerous interspecific hybrids, both among the diploid (*P. cerasifera, P. salicina* Lindl.) and the hexaploid (*P. domestica, P. insititia*) plums, as well as other combinations with different ploidy levels.

1. Myrobalan Plums (*P. cerasifera* Ehrh). There is a wider availability of plum rootstocks for fresh fruit production (*P. domestica* and *P. salicina*), than for other species of the *Prunus* species. However, the considerable incompatibility of many varieties grafted onto Myrobalan and Marianna rootstocks is well known (Herrero, 1951, 1962; Cambra and Cambra, 1973; Moreno et al., 1995c), and these are often the most frequently used rootstocks for plum growing. Interstocks have been used to overcome incompatibility problems of some varieties with these rootstocks (Tabuenca et al., 1991). However, the use of interstocks increases tree production costs and therefore, the initial investment.

A selection programme of Myrobalan clones was initiated in 1950 at the EEAD-CSIC. A large population of Myrobalan plum seedlings was obtained commercially from Spanish sources. In an initial selection phase, their aptitude for vegetative propagation was evaluated, and 26 clones of the best performers were retained. Compatibility studies in the nursery with plum and apricot varieties led to the pre-selection of a limited number of clones, some of which showed good compatibility with plum varieties that were incompatible when grafted onto 'Myrobalan B' and other Myrobalan and Marianna rootstocks (Cambra and Cambra, 1972; 1973). Likewise, improved compatibility with apricot was observed for some of the clones under selection, in comparison with other commercial rootstocks (Cambra, 1979a; 1990a). However, good compatibility with apricot could not be guaranteed, especially for the varieties 'Búlida', 'Canino' and 'Moniquí', included in the difficult-to-graft non congenial cultivars (Crossa-Raynaud and Audergon, 1987).

Among the pre-selected clones, 'Ademir' (Myrobalan 599 AD) was outstanding (Cambra, 1979a, 1990a, Moreno et al., 1995c), as it propagated readily by hardwood cuttings, and had tolerance to root asphyxia and iron-induced chlorosis in compact and calcareous soils. It also had good production characteristics, when grafted with European plum varieties ('Reina Claudia de Bavay' and 'Reina Claudia Tardía de Chambourcy'). Furthermore, it was found to be resistant to root-knot nematodes, *Meloidogyne* spp. (Pinochet et al., 1999; D. Esmenjaud, personal communication). This interest led to a request for Plant Variety Rights to be processed in the Community Plant Variety Office (CPVO), which were obtained in 2002.

From 1978 the performance of 'Adara' (plum 2977 AD) was studied as a rootstock for different stone fruit species. Due to its morphological characteristics (Moreno, 1989a), 'Adara' could be included in the fast-growing plum group described by Bernhard and Grasselly (1959). It originates from a population of open-pollinated Myrobalan plums. As characteristic of this group, 'Adara' adapts well to heavy and calcareous soils, where root asphyxia and iron-induced chlorosis are frequent problems. Moreover, it is easily propagated by hardwood cuttings and is graft compatible with a large number of cherry varieties (Moreno and Tabuenca, 1991; Moreno et al, 1995b). It also performs well with some varieties of peach, plum and apricot (Tabuenca and Moreno, 1988; Moreno and Tabuenca, 1991; Moreno et al., 1995b; 1996) and is resistant to root-knot nematodes (Pinochet et al., 1999).

The interest in Myrobalans as rootstocks for plum and other stone fruit species is due to their high tolerance to root asphyxia, iron-induced chlorosis and salinity; as well as their resistance to root-knot nematodes, soil borne pathogens (fungi and bacteria) and

phytoplasms (Rowe and Catlin, 1971; Dosba, 1992; El-Motaium et al., 1994; Esmenjaud et al., 1994). These tolerances suggest that they could be used as a gene source for obtaining new rootstocks. Furthermore, they are easy to hybridise within the *Prunus* group (Casas et al., 1999), thereby opening up the possibility of obtaining complex hybrids (Moreno et al., 1999).

Thus, in 1997 a new breeding programme for rootstocks was initiated for peach and other stone fruit species. Controlled interspecific crosses were undertaken with the purpose of bringing together the desirable traits of plum species (mainly *P. cerasifera*), the peach species, and/or the species closely-related to peach [*P. persica*, *P. amygdalus* and *P. davidiana* (Carr.) Franch.] (Moreno et al., 1999). Although peach is generally incompatible when grafted onto *P. cerasifera* or Myrobalan species (Herrero, 1951, 1955; Tabuenca, 1960, 1962), there are Myrobalan clones compatible with numerous peach varieties (Tabuenca and Moreno, 1988; Moreno et al., 1993; 1994a; 1995b). These can be used as a means to obtain two-way or more cross hybrids, to produce complex hybrids. At present, there are progeny of these types of interspecific hybrids in the selection phase. Apart from good compatibility with peach and other stone fruit species, multi-tolerance and/or resistance to the primary limiting soil factors (e.g., chlorosis, asphyxia, nematodes, replant disorders) found in the Mediterranean area are considered priority objectives in this programme. Micropropagation of some of these progeny has accelerated the selection process, thereby reducing the time needed to have a sufficient number of plants available. This has advanced the breeding programme to the initial screening phases for tolerance or resistance to chlorosis, asphyxia, nematodes, bacteria and for rootstock-variety compatibility.

Following the same objectives, another rootstock breeding programme has recently been implemented for apricot. Interspecific crosses were made between the plum rootstock Myrobalan and an exigent apricot variety (‘Moniquí’), with the intention of incorporating traits related to adaptation to the heavy soils of the Ebro Valley from the Myrobalan rootstock (Daorden et al., 2001). Application of in vitro culture techniques, such as embryo recovery and micropropagation, have also reduced the time needed to obtain new hybrid clones as well as their multiplication; thereby, increasing the number of plants for study. Additionally more clones can be developed, since seeds can now be recovered from embryos that would otherwise not be viable using the traditional techniques (García et al., 2001).

2. ‘Pollizo de Murcia’ (*P. insititia* L.) and Other Slow-growing Plums (*P. domestica* L.).

In Spain, the ‘Pollizo de Murcia’ plum is the most common rootstock for stone fruit production in the region of Murcia (southeastern Spain). This area is one of the largest plum producing areas in the country. ‘Pollizo de Murcia’ offers tolerance to chlorosis, to root asphyxia, and to salinity (Cambra, 1970). In its area of origin, it was propagated using root suckers, which can lead to problems of disease and virus transmission, and die-back (Llácer et al., 1986).

Due to the interest in this rootstock, its cultural problems, and the need for a rational and economical propagation system, clonal selection began at the EEAD-CSIC in 1963. Selection of ‘Pollizo de Murcia’ was initiated with the collection of this material in different parts of the province of Murcia, and then evaluation of its aptitude to vegetative multiplication and nursery performance. As a result of this process, some clones were selected although some problems still persisted, such as a poor ability to vegetative propagate by cuttings (Cambra, 1970; 1979b; Moreno, 1989b). The best-performing clones were further studied for compatibility in the nursery, by grafting with peach, plum and apricot varieties and then followed by orchard trials (Moreno, 1990; Moreno et al., 1990).

From these Pollizo selections, ‘Adesoto’ (formerly ‘Puebla de Soto 101’) was selected, which is a multi-purpose rootstock for different stone fruit species, especially for peach grown in heavy and calcareous soil conditions (Moreno, 1991a; Moreno et al., 1995a; Moreno and Cambra, 1998). Field data demonstrate its high productive performance and improved fruit quality as a rootstock, as well as its reduction of scion

vigour, between 30 and 40% in comparison to GF 677, and its tolerance to iron-induced chlorosis and root asphyxia (Moreno et al., 1990; Iglesias et al., 2001). This rootstock is also resistant to several species of root-knot nematodes (*M. arenaria, M. javanica, M. incognita*) (Pinochet et al., 1991; 1999). Interest in 'Adesoto' led to a request for Plant Variety Rights to be processed in the Community Plant Variety Office (CPVO), which were obtained in 2002. At present, it is at the commercial phase in Spain and other countries of the Mediterranean area, and it is beginning to be marketed in Chile and will soon be available in other American countries.

On the other hand, due to the lack of diversity observed in the initial population (Cambra, 1979b), it was necessary to use the genetic variability found in sexual reproduction by seed, following open-pollination of mature Pollizo trees. The study began with seed collection and after the initial work, 153 clones were chosen, from which a new field of mother-plants was established in 1980. This material was used to make a pre-selection of 4 clones (Moreno, 1990; 1991b). These clones were observed to have a good aptitude for vegetative propagation and nursery performance when grafted with varieties. Resistance to nematodes has also been observed (Pinochet et al., 1999; D. Esmenjaud, personal communication). At the same time, in the Department of Fruit Culture in the Service of Agro-Food Research of the Regional Government of Aragon (SIA-DGA), two clonal Pollizos, 'Montizo' and 'Monpol', were also selected from a seed population of open-pollinated 'Pollizos de Murcia' (Felipe et al., 1989, Felipe and Pascual, 1990). Therefore, joint evaluation studies of these materials are currently being conducted in orchard trials.

Collections from other autochthonous *P. domestica* populations that were used as rootstocks for peach in different Spanish locations and their subsequent study at the EEAD-CSIC have led to the following findings: tolerance to chlorosis and resistance to nematodes in two common plums called 'Constantí' and 'Mas Rubí' (Cambra et al., 1989; Moreno et al., 2001a; Pinochet et al., 1999). These rootstocks are presently in the evaluation phase in field trials.

Selection of Peach × Almond Hybrid Rootstocks

The peach and almond hybrids [*Prunus amygdalo-persica* (West) Rehd.] are primarily used in calcareous soils since they tolerate chlorosis well and are generally graft compatible with peach and almond. They are also vigorous and therefore, appropriate for use in poor, dry soils and in fruit tree replanting situations (Bernhard and Grasselly, 1959; Kester and Assay, 1986; Byrne et al., 1990; Socias i Company et al., 1995; Iglesias et al., 2001). However, some of the significant drawbacks of these rootstocks are sensitivity to root asphyxia in heavy soils, sensitivity to certain nematodes and soil borne fungi, excess vigour conferred to the grafted varieties, and decreased fruit size and colour when compared to other plum rootstocks or peach seedlings (Loreti and Massai, 1990).

At the EEAD-CSIC, selection of peach × almond hybrids began in 1970 with the identification and collection of 58 spontaneous hybrids, collected in eleven provinces of Spain, which were incorporated into the *Prunus* collection at EEAD-CSIC. Work basically focused on studying their aptitude for vegetative propagation (Cambra, 1979c). For the outstanding clones in the first phase, their sanitary status (i.e., virus, viroid, mycoplasma) was determined and propagation conditions were optimised, seeking to control the vigour of the seedling with a view to grafting (Cambra, 1981). In experimental nurseries, their compatibility was determined with a given number of varieties as well as their tolerance to chlorosis. Later, the most outstanding clones were established in trials in order to evaluate their influence on the productive characteristics of the varieties grafted.

As a consequence of this long selection process, the rootstock 'Adafuel' (formerly Jarafuel) was reported to have the best vegetative propagation characteristics, compatibility with peach and almond, tolerance to chlorosis and high vigour (Cambra, 1981, 1990b; Cambra and Iturrioz, 1986; Moreno et al., 1994b). A high level of vigour is recommended for poor soils with problems of chlorosis (Moreno et al., 1995d). Unfortunately, 'Adafuel' was highly susceptible to root-knot nematodes.

More recently, ‘Adarcias’ (formerly Arbucias), a natural peach-almond hybrid, was selected for its good performance with peach varieties, conferring less vigour than the ‘GF 677’ and ‘Adafuel’ hybrids. Furthermore, it induces increased productivity and better fruit quality (Moreno and Cambra, 1994; Moreno et al., 1994b; Albás et al., 2002). These characteristics make it more appropriate for its use in fertile soils that require greater control of tree vigour, allowing an increase in the density of the plantation. As in the case of ‘Adafuel’, an application to obtain its protection in the Spanish Register for varieties was submitted for ‘Adarcias’ and recently obtained.

Given the interest in the performance of ‘Adafuel’ and ‘Adarcias’ rootstocks and the new selections ‘Garnem’, ‘Monegro’ and ‘Felinem’ (from the cross between Garfi almond × Nemared peach, or GxN) developed at the SIA-DGA (Felipe et al., 1997; Pinochet et al., 1992; Socias i Company et al., 1995; Gómez Aparisi et al., 2001), a trial was established to evaluate the agronomic characteristics of these rootstocks, with special reference to the influence on the vigour, nutritional status, yield efficiency and fruit quality of the grafted varieties.

Selection of Rootstocks for Cherry

Cherry has been a fruit species particularly affected by the lack of adequate rootstocks. Those available do not perform well and experience serious limitations, such us poor adaptation to heavy soils, root asphyxia problems, chlorosis in calcareous soils, sensitivity to drought and excessive vigour conferred to grafted varieties.

In Spain and other Mediterranean countries, the rootstock ‘St. Lucie’ (*P. mahaleb* L.) or a clonal selection, ‘SL 64’, have been widely used as rootstocks for non-irrigated cherries, or in well-drained and calcareous soils. However, the rootstock ‘St. Lucie’ does not adapt well to heavy soils in waterlogged conditions (Breton et al., 1972; Perry, 1987; Moreno et al., 1996).

The sour cherry (*P. cerasus* L.) is another species used as a cherry rootstock for its positive characteristics such as reduction in vigour in comparison to cherry seedlings, early production and good adaptation to heavy soils (Moreno et al., 2001b). However, this species has some problems, such as excess suckering and lack of graft affinity with some of the sweet cherry varieties.

In 1969, at the EEAD-CSIC, a selection process was begun for local *P. cerasus* varieties as rootstocks for cherry (Cambra, 1979d). Among these varieties was the ‘Masto de Montañana’ population. Following propagation studies, 5 clones were pre-selected, including the 'Masto de Montañana 9’. However, the inaptitude for vegetative propagation using hardwood cuttings led to ‘in vitro’ culture techniques to be implemented. Furthermore, excess suckering is still a serious problem for orchard management. The use of in vitro culture techniques, conducted at SIA-DGA, permitted a more practical propagation method to allow the selection process to continue (Gella and Marín, 1990; Marín and Gella, 1991).

As mentioned in the section on plum rootstocks, the performance of ‘Adara’ has been studied since 1978 as a rootstock for different stone fruits, but especially for cherry, due to its compatibility with a large number of varieties of this species (Moreno and Tabuenca, 1991; Moreno et al., 1995b). ‘Adara’ is a vigorous clone that allows cherry to be grown under irrigation (Moreno, 1989; Tabuenca and Moreno, 1988; Moreno and Tabuenca, 1991; Moreno et al., 1995b; 1996). The orchard performance of ‘Adara’ as a cherry rootstock has been compared with some of the most widely used rootstocks for cherry. Under irrigation, in heavy and calcareous soils, trees grafted onto ‘Adara’ have yielded the most, and neither dead trees nor chlorosis were observed. On the other hand, under the same conditions, high mortality rate was observed in trees grafted on St. Lucie ‘SL 64’ (over 50%) and chlorosis, nutritional deficiencies and low productivity were observed in trees grafted on Colt (Moreno et al., 1996).

The performance of the plum ‘Adara’ as a multi-purpose rootstock for different stone fruit species is outstanding, especially for cherries growing under irrigated conditions, and in heavy and calcareous soils with nematode problems (Moreno, 1989a;

Moreno and Tabuenca, 1991; Moreno et al., 1995b; Pinochet et al., 1999). Plant Variety Rights were requested and obtained from the Community Plant Variety Office in 2002. 'Adara' is presently in the commercial stage of development.

Present-Day Perspectives

At present, the breeding programme for *Prunus* rootstocks at EEAD-CSIC focuses mainly on obtaining new rootstocks adapted to the limiting conditions of the Mediterranean region (Moreno et al., 1999). Among these limitations, chlorosis, root asphyxia, replant disorders, presence of nematodes, and soil fungi are the most common and important. Rootstocks in the selection and evaluation process include the peach × almond hybrid rootstocks (*P. amygdalo-persica*), 'Pollizo de Murcia' plums (*P. insititia*), European plums (*P. domestica*), Myrobalans (*P. cerasifera*), other hybrids within the *Prunus* genus and peach seedlings (*P. persica*). Furthermore, the existing genetic variability of the *Prunus* genus is being used to obtain new rootstocks through interspecific crosses.

Evaluations are primary based on ease of vegetative propagation, growth performance in the nursery, rootstock-variety compatibility, and use with multiple fruit species as well as tolerance to the most important stresses under our conditions (e.g., calcareous and heavy soils, with problems of chlorosis and root asphyxia). In order to evaluate the possible influence of the rootstock on scion production characteristics and fruit quality, tree vigour and yield are determined as well as fruit weight and size, colour, firmness, acidity, pH, soluble solid concentration, main sugar content and fruit ripening index.

In a fruit tree breeding programme, the greatest limiting factor is the capacity to evaluate trees in the field. This is due both to the long time needed for progeny from crosses to complete their vegetative and reproductive development, and the land area required to grow the seedling populations and selections.

At present, the new possibilities of multiplication that in vitro culture can offer have reduced costs in certain cases, and have also favoured more widespread use of new rootstocks that are difficult to propagate using traditional techniques. Furthermore, with the traditional propagation systems several years are needed to have enough mother-plants and seedlings available for evaluation. With in vitro propagation, this phase can be shortened in time and in space thus opening up new perspectives to speed up the selection and breeding processes, which are inherently slow in arboreal species.

In this regard, to avoid the high economic cost and time needed to work with woody fruit species, a new method has been developed to select new *Prunus* rootstocks tolerant to iron-chlorosis (Gogorcena et al., 2000). Thus, plants from in vitro culture with only a few weeks of development can undergo induced iron deficiency in hydroponic culture conditions and their capacity to reduce iron compounds can be analysed as a screening technique for chlorosis. For the genotypes studied to date, the results observed in field chlorosis-inducing conditions seem promising (Jiménez et al., 2003).

The morphological characterisation of rootstocks selected or in the selection phase at the EEAD-CSIC has traditionally been carried out by monitoring the traits proposed in the descriptions provided by the UPOV and IPGRI guidelines. More recently, molecular characterisation has been carried out using RAPDs (Casas et al., 1999) and SSRs (Bouhadida et al., 2004).

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